

Marked up version showing changes to specification under 37 C.F.R. § 1.121(b)



At page 1, after the Title, please insert the following paragraph:

RELATEDNESS OF THE APPLICATION

This application is a divisional of U.S. Application Serial No. 08/973,124, filed May 11, 1998, which is a 35 U.S.C. § 371 national phase application of International Application No. PCT/US96/08014, filed May 30, 1996. International Application No. PCT/US96/08014 is a continuation-in-part of U.S. Application Serial No. 08/465,594, filed June 5, 1995, now Pat. No. 5,846,713, a continuation-in-part of 08/465,591, filed June 5, 1995, now Pat. No. 5,837,834, a continuation-in-part of U.S. Application Serial No. 08/479,725, filed June 7, 1995, now Pat. No. 5,674,685, a continuation-in-part of U.S. Application Serial No. 08/479,783, filed June 7, 1995, now Pat. No. 5,668,264, and a continuation-in-part of U.S. Application Serial No. 08/618,693, filed March 20, 1996, now Pat. No. 5,723,594. PCT/US96/08014 also claims priority from U.S. Application Serial No. 08/458,423, filed June 2, 1995, now Pat. No. 5,731,144, and U.S. Application Serial No. 08/458,424, filed June 2, 1995, now Pat. No. 5,731,424.

At page 3, please replace the paragraph beginning at line 3 with the following:

A number of diseases have been associated with TGFβ1 overproduction. Fibrotic diseases associated with TGFβ1 overproduction can be divided into chronic conditions such as fibrosis of kidney, lung and liver and more acute conditions such as dermal scarring and restenosis. Synthesis and secretion of TGFβ1 by tumor cells can also lead to immune suppression such as seen in patients with aggressive brain or breast tumors (Arteaga *et al.*, (1993) *J Clin Invest* 92: 2569-2576). The course of ~~Leishmanial~~ Leishmanial infection in mice is drastically altered by TGFβ1 (Barral-Netto *et al.*, (1992) *Science* 257:545-547). TGFβ1 exacerbated the disease, whereas TGFβ1 antibodies halted the progression of the disease in genetically susceptible mice. Genetically resistant mice became susceptible to ~~Leshmanial~~ Leishmanial infection upon administration of TGFβ1.

At page 5, please replace the paragraph beginning at line 13 with the following:

High plasma concentrations of TGFβ1 have been shown to indicate poor prognosis for advanced breast cancer patients (Anscher *et al.*: (1993) *N Engl J Med* 328:1592-8). Patients with

high circulating TGF β before high dose chemotherapy and autologous bone marrow transplantation are at high risk for hepatic veno-occlusive disease (15-50% of all patients with a mortality rate up to 50%) and idiopathic interstitial pneumonitis (40-60% of all patients). The implication of these findings is 1) that elevated plasma levels of TGF β 1 can be used to identify at risk patients and 2) that reduction of TGF β 1 could decrease the morbidity and mortality of these common treatments for breast cancer patients.

At page 7, please replace the paragraph beginning at line 13 with the following:

In view of its importance in proliferative disease states, antagonists of PDGF may find useful clinical applications. Currently, antibodies to PDGF (Johnsson *et al.*, (1985) Proc. Natl. Acad. Sci., U. S. A. 82: 1721-1725; Ferns *et al.*, (1991) Science 253: 1129-1132; Herrenet *et al.*, (1993) Biochimica et Biophysica Acta 1173, 494 204-302) and the soluble PDGF receptors (Herren *et al.*, (1993) Biochimica et Biophysica Acta 1173: 494 204-302; Duan *et al.*, (1991) J. Biol. Chem. 266: 413-418; Teisman *et al.*, (1993) J. Biol. Chem. 268: 9621-9628) are the most potent and specific antagonists of PDGF. Neutralizing antibodies to PDGF have been shown to revert the SSV-transformed phenotype (Johnsson *et al.*, (1985) Proc. Natl. Acad. Sci., U. S. A. 82: 1721-1725) and to inhibit the development of neointimal lesions following arterial injury (Ferns *et al.*, (1991) Science 253: 1129-1132). Other inhibitors of PDGF such as suramin (Williams *et al.*, (1984) J. Biol. Chem. 259: 5287-5294; Betsholtz *et al.*, (1984) Cell 39 447-457), neomycin (Vassbotn *et al.*, (1992) J. Biol. Chem. 267 15635-15641) and peptides derived from the PDGF amino acid sequence (Engström *et al.*, 1992) J. Biol. Chem. 267: 16581-16587) have been reported, however, they are either too toxic or lack sufficient specificity or potency to be good drug candidates. Other types of antagonists of possible clinical utility are molecules that selectively inhibit the PDGF receptor tyrosine kinase (Buchdunger *et al.*, (1995) Proc. Natl. Acad. Sci., U. S. A. 92: 2558-2562; Kovalenko *et al.*, (1994) Cancer Res. 54: 6106-6114).

At page 8, please replace the paragraph beginning at line 5 with the following:

Human Keratinocyte Growth Factor (hKGF) is a small (26-28KD) basic heparin-binding growth factor and a member of the FGF family. hKGF is a relatively newly identified molecule,

which is also known as FGF-7 (Finch *et al.*, (1989) Science **244** 245:752-755). It is a growth factor specific for epithelial cells (Rubin *et al.*, (1989) Proc Natl Acad Sci USA **86**:802-806), and its main function is in development/morphogenesis (Werner *et al.*, (1994) Science **266**:819-822) and in wound healing (Werner *et al.*, (1992) Proc Natl Acad Sci USA **89**:6896-6900). The major *in vivo* source of hKGF is stromal fibroblasts (Finch *et al.*, (1989) Science **244** 245:752-755). Microvascular endothelial cells (Smola *et al.*, (1993) J Cell Biol **122**:417-429) and very recently, activated intraepithelial gd T cells (Boismenu *et al.*, (1994) Science **266**:1253-1255) have also been shown to synthesize hKGF. hKGF expression is stimulated in wounds (Werner *et al.*, (1992) Proc Natl Acad Sci USA **89**:6896-6900). Several cytokines are shown to be hKGF inducers (Brauchle *et al.*, (1994) Oncogene **9**:3199-3204), with IL-1 the most potent one (Brauchle *et al.*, (1994) Oncogene **9**:3199-3204; Chedid *et al.*, (1994) J Biol Chem **269**:10753-10757). Unlike bFGF, hKGF has a signal peptide and thus is secreted by producing cells (Finch *et al.*, (1989) Science **244** 245:752-755). hKGF can be overexpressed in *E. coli* and the recombinant protein (~19-21 KD) is biologically active (Ron *et al.*, (1993) J Biol Chem **268**:2984-2988). The *E. coli* derived recombinant protein is 10 times more mitogenic than the native protein (Ron *et al.*, (1993) J Biol Chem **268**:2984-2988). This difference may be due to glycosylation. The native protein has a potential Asn glycosylation site (Ron *et al.*, (1993) J Biol Chem **268**:2984-2988).

At page 11, please replace the paragraph beginning at line 15 with the following:

In vivo, hKGF causes mammary duct dilation and rampant epithelial hyperplasia, both of which are common features of breast cancers (Ulich *et al.*, (1994) Am J Pathol **144**:862-868; Yi *et al.*, (1994) Am J Pathol **145**:1015-1022). However, the ductal epithelium of breastfeeding rats is resistant to the growth promoting effects of hKGF and this is of interest in regard to epidemiological observations that pregnancy in women decreases susceptibility to breast cancer and that dairy cows almost never develop breast cancer (Kuzma, 1977, *Breast in Pathology*, Mosby Co.). There is additional supporting evidence implicating hKGF in breast cancer. hKGF mRNA has been detected recently in normal human breast tissue and in 12 of 15 breast tumor samples tested (Koos *et al.*, (1993) J Steroid Biochem Molec Biol **45**:217-225). The presence of hKGF mRNA in breast tumors considered in conjunction with the observation that hKGF is present in nonneoplastic mammary

glands and that hKGF causes rampant proliferation of mammary epithelium suggests that hKGF may be an autocrine or paracrine growth factor important in the regulation of the growth of normal and neoplastic mammary epithelium (Ulich *et al.*, (1994) Am J Pathol 144:862-868). Infiltrating ductal mammary adenocarcinoma is characteristically enveloped by a ~~desmoplastic~~ desmoplastic stroma that has been postulated to represent a defensive host response to the carcinoma (Ulich *et al.*, (1994) Am J Pathol 144:862-868). Since hKGF is stroma derived it is possible that the desmoplastic stroma contributes rather than inhibits the growth of the tumor.

At page 12, please replace the paragraph beginning at line 6 with the following:

The growth promoting effect of androgens on prostate tumors appears to be mediated through hKGF (Yan *et al.*, (1992) Mol Endo 6:2123-2128), as androgens induce the expression of hKGF in prostate stroma cells. Prostate tumors that are androgen dependent *in vivo*, are androgen independent *in vitro*, but hKGF dependent (Yan *et al.*, (1992) Mol Endo 6:2123-2128). In agreement with the role of hKGF as andromedin is the observation that hKGF functions in epithelial induction during seminal vesicle development, a process that is directed by androgen (Alarid *et al.*, (1994) Proc Natl Acad Sci USA 91:1074-1078). Furthermore, hKGF causes aberrant activation of the androgen receptor, thus probably contributing to the failure of androgen ablation therapy in prostate cancer (Culig *et al.*, (1994) Cancer Res 54:5474-5478). Based on this information, it is possible that genetic alterations cause hKGF to escape androgen regulation and thus convert the androgen dependent tumor into an androgen independent, highly malignant tumor. Such tumors would still be able to express the androgen regulated marker PSA, as hKGF also causes the aberrant activation of the androgen receptor. It is also likely that hKGF might be responsible for Benign Prostate Hypertrophy (BPH), a common health problem in older men (D. Bottaro, personal ~~communication~~ communication).

At page 13, please replace the paragraph beginning at line 4 with the following:

Blocking the interaction of growth factors and lymphokines with their cell surface receptor using antagonists has been an approach for disease treatment. The discovery of such antagonists requires the availability of biochemical assays for the receptor-growth factor or lymphokine interaction. A classic assay has been the competitive inhibition of radiolabeled growth factor or

lymphokine (tracer) to its cell surface receptor. These types of assays utilize cell lines that express the relevant receptor on their surface and ~~determines~~ determine the amount of cell bound tracer in the presence of various concentrations of potential antagonists. Additionally, other assays utilize membrane extracts from cell lines that express the relevant receptor, and tracer binding is followed by filter binding (see Nenquest Drug Discovery System: Human Tumor Necrosis Factor-Alpha, NEN Research Products, E. I. ~~DePont~~ DuPont de Nemours & Co. (Inc.), Boston, MA) or by immobilizing the membrane extracts onto solid supports (Urdal *et al.*, (1988) J Biol Chem 263:2870-2877; Smith *et al.*, (1991) Bioch Bioph Res Comm 176:335-342). Receptor induced electrophoretic mobility shift of tracer has been applied to identify the presence and size of cell surface receptors by crosslinking the receptor to the tracer and then analyzing on denaturing gels (for example see Kull *et al.*, (1985) Proc natl Acad Sci USA 82:5756-5760; Hohmann *et al.*, (1989) J Biol Chem 264:14927-14934; Stauber *et al.*, (1989) J Biol Chem 264:3573-3576). The use of native gels and non-crosslinked complexes has not been described for growth factors or lymphokines and their receptors, but has been widely applied to study nucleic acid protein interactions (Sambrook *et al.*, (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

At page 14, please replace the paragraph beginning at line 5 with the following:

A method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules has been developed. This method, Systematic Evolution of Ligands by EXponential enrichment, termed SELEX, is described in United States Patent Application Serial No. 07/536,428, entitled "Systematic Evolution of Ligands by Exponential Enrichment," now abandoned, United States Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands," now U.S. Patent No. 5,475,096, and United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled "Methods for Identifying Nucleic Acid Ligands," now United States Patent No. 5,270,163 (see also ~~PCT/US91/04078~~ WO 91/19813), each of which is herein specifically incorporated by reference. Each of these applications, collectively referred to herein as the SELEX Patent Applications, describes a fundamentally novel method for making a nucleic acid ligand to any desired target molecule.

At page 15, please replace the paragraph beginning at line 1 with the following:

The basic SELEX method has been modified to achieve a number of specific objectives. For example, United States Patent Application Serial No. 07/960,093, filed October 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of Structure," describes the use of SELEX in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. United States Patent Application Serial No. 08/123,935, filed September 17, 1993, entitled "Photoselection of Nucleic Acid Ligands" describes a SELEX based method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine," describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed "Counter-SELEX." United States Patent Application Serial No. 08/143,564, filed October 25, 1993, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Solution SELEX," now abandoned (see U.S. Pat. No. 5,567,588), describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. United States Patent Application Serial No. 07/964,624, filed October 21, 1992, entitled "~~Methods of Producing~~ Nucleic Acid Ligands to HIV-RT and HIV-1 Rev," now U.S. Pat. No. 5,496,938, describes methods for obtaining improved nucleic acid ligands after SELEX has been performed. United States Patent Application Serial No. 08/400,440, filed March 8, 1995, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX," now U.S. Pat. No. 5,705,337, describes methods for covalently linking a ligand to its target.

At page 15, please replace the paragraph beginning at line 25 with the following:

The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified nucleic acid ligands containing modified nucleotides are described in United States Patent

Application Serial No. 08/117,991, filed September 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," now abandoned (see U.S. Pat. No. 5,660,985), that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. United States Patent Application Serial No. 08/134,028, *supra*, describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH₂), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). United States Patent Application Serial No. 08/264,029, filed June 22, 1994, entitled "Novel Method of Preparation of ~~2'-Modified Pyrimidine~~ Known and Novel 2'-Modified Nucleosides by Intramolecular Nucleophilic Displacement," describes oligonucleotides containing various 2'-modified pyrimidines.

At page 16, please replace the paragraph beginning at line 11 with the following:

The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in United States Patent Application Serial No. 08/284,063, filed August 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Chimeric SELEX," now U.S. Pat. No. 5,637,459, and United States Patent Application Serial No. 08/234,997, filed April 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX," now U.S. Pat. No. 5,683,867, respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules. Each of the above described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

At page 18, please replace the paragraph beginning at line 12 with the following:

In addition, the present invention includes the RNA ligands to hKGF identified according to the above-described method, including those ligands shown in Tables 16 and 23 (SEQ ID NOS:189-~~262~~ 264, ~~272~~ 268-304). Also included are RNA ligands to hKGF that are substantially homologous to any of the given ligands and that have substantially the same ability to bind hKGF and inhibit the interaction of hKGF with its receptor. Further included in this invention are nucleic acid ligands to hKGF that have substantially the same structural form as the ligands presented herein and that have

substantially the same ability to bind hKGF and inhibit the interaction of hKGF with its receptor.

At page 19, please replace the paragraph beginning at line 9 with the following:

Further included in this invention is a method for assaying cells to determine whether they express a growth factor plasma membrane bound receptor comprising the steps of (a) solubilizing the cells; (b) creating a plasma membrane extract of the cells; (c) reacting the plasma membrane extract with a labeled growth factor; (d) analyzing the reaction between the plasma membrane extract with the labeled growth factor by electrophoresis under native conditions; (e) comparing the electrophoresis of step (d) with electrophoresis of labeled growth factor; and (f) visualizing the results of the electrophoresis to determine whether a complex is formed with altered mobility relative to the mobility of a labeled growth factor alone.

At page 20, please replace the paragraph beginning at line 1 with the following:

Figures 5A, 5B and 5C show the binding of minimal high affinity DNA ligands to PDGF. The fraction of ^{32}P 5' end-labeled DNA ligands bound to varying concentrations of PDGF was determined by the nitrocellulose filter binding method. Minimal ligands tested were 20t (o), 36t (Δ), and 41t (\blacksquare) (\square). Oligonucleotide concentrations in these experiments were ≈ 10 pM (PDGF-AB and PDGF-BB) and ≈ 50 pM (PDGF AA). Data points were fitted to eq. 1 (for binding of the DNA ligands to PDGF-AA) or to eq. 2 (for binding to PDGF AB and BB) using the non-linear least squares method. Binding reactions were done at 37°C in binding buffer (PBSM with 0.01% HSA).

At page 20, please replace the paragraph beginning at line 10 with the following:

Figure 6 shows the dissociation rate determination for the high affinity interaction between the minimal DNA ligands and PDGF AB. The fraction of $5' ^{32}\text{P}$ end-labeled ligands 20t (o), 36t (Δ), and 41t (\blacksquare) (\square), all at 0.17 nM, bound to PDGF AB (1 nM) was measured by nitrocellulose filter binding at the indicated time points following the addition of a 500-fold excess of the unlabeled competitor. The dissociation rate constant (k_{off}) values were determined by fitting the data points to eq. 3. The experiments were performed at 37°C in binding buffer.

At page 20, please replace the paragraph beginning at line 18 with the following:

Figure 7 shows the effect of DNA ligands on the binding of ^{125}I -PDGF-BB and ^{125}I -PDGF-AA to PDGF α -receptors expressed in PAE cells.

At page 22, please replace the paragraph beginning at line 14 with the following:

This application describes high-affinity nucleic acid ligands to TGF β , PDGF, and hKGF identified through the method known as SELEX. SELEX is described in U.S. Patent Application Serial No. 07/536,428, entitled Systematic Evolution of Ligands by EXponential Enrichment, now abandoned, U.S. Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled Nucleic Acid Ligands, now U.S. Patent No. 5,475,096 and United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled Methods for Identifying Nucleic Acid Ligands, now United States Patent No. 5,270,163, (see also PCT/US91/04078 WO 91/19813). These applications, each specifically incorporated herein by reference, are collectively called the SELEX Patent Applications.

At page 24, please replace the paragraph beginning at line 4 with the following:

The nucleic acid ligands described herein can be complexed with a lipophilic compound (e.g., cholesterol) or attached to or encapsulated in a complex comprised of lipophilic components (e.g., a liposome). The complexed nucleic acid ligands can enhance the cellular uptake of the nucleic acid ligands by a cell for delivery of the nucleic acid ligands to an intracellular target. U.S. Patent Application No. 08/434,465, filed May 4, 1995, entitled "Nucleic Acid Ligand Complexes," now U.S. Patent No. 6,011,020, which is incorporated in its entirety herein, describes a method for preparing a therapeutic or diagnostic complex comprised of a nucleic acid ligand and a lipophilic compound or a non-immunogenic, high molecular weight compound.

At page 25, please replace the paragraph beginning at line 15 with the following:

In co-pending and commonly assigned U.S. Patent Application Serial No. 07/964,624, filed October 21, 1992 ('624), now U.S. Patent No. 5,496,938, methods are described for obtaining improved nucleic acid ligands after SELEX has been performed. The '624 application, entitled Methods of Producing Nucleic Acid Ligands to HIV-RT and HIV-1 Rev, is specifically incorporated

herein by reference. Further included in this patent are methods for determining the three-dimensional structures of nucleic acid ligands. Such methods include mathematical modeling and structure modifications of the SELEX-derived ligands, such as chemical modification and nucleotide substitution.

At page 26, please replace the paragraph beginning at line 10 with the following:

In the present invention, a SELEX experiment was also performed in search of RNA ligands with specific high affinity for hKGF from degenerate libraries containing 40 random positions (40N) (Table 14). This invention includes the specific RNA ligands to hKGF shown in Tables 16 and 23 and Figure 12 (SEQ ID NOS:189-262, ~~272~~ 268-304), identified by the methods described in Examples 16 and 17. This invention further includes RNA ligands to hKGF which inhibit the interaction of hKGF with its receptor.

At page 26, please replace the paragraph beginning at line 17 with the following:

The scope of the ligands covered by this invention extends to all nucleic acid ligands of TGF β , PDGF, and hKGF, modified and unmodified, preferably those identified according to the SELEX procedure. More specifically, this invention includes nucleic acid sequences that are substantially homologous to the ligands shown in Tables 3, 6, 8, 9, 13, 16, and 23 and Figures 3, 4, ~~and 9 and 12~~ (SEQ ID NOS:12-42, 55-89, 93-124, 128-176, 189-262, ~~272~~ 268-304). By substantially homologous it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. A review of the sequence homologies of the nucleic acid ligands shown in Tables 3 and 6 (SEQ ID NOS:12-42, 55-89) for TGF β , Tables 8 and 13 (SEQ ID NOS:93-124, 128-170) for PDGF, and Tables 16 and 23 (SEQ ID NOS:189-262, 272-304) for hKGF shows that sequences with little or no primary homology may have substantially the same ability to bind a given target. For these reasons, this invention also includes nucleic acid ligands that have substantially the same structure and ability to bind TGF β , PDGF, and hKGF as the nucleic acid ligands shown in Tables 3, 6, 8, 9, 13, 16, and 23 and Figures 3, 4, ~~and 9 and 12~~ (SEQ ID NOS:12-42, 55-89, 93-124, 128-176, 189-262, ~~272~~ 268-304). Substantially the same structure for PDGF includes all nucleic acid ligands having the common structural elements shown in Figure 3 that lead to the affinity to PDGF.

Substantially the same ability to bind TGF β , PDGF, or hKGF means that the affinity is within one or two orders of magnitude of the affinity of the ligands described herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence -- substantially homologous to those specifically described herein -- has substantially the same ability to bind TGF β , PDGF, or hKGF.

At page 27, please replace the paragraph beginning at line 10 with the following:

This invention also includes the ligands as described above, wherein certain chemical modifications are made in order to increase the *in vivo* stability of the ligand or to enhance or mediate the delivery of the ligand. Examples of such modifications include chemical substitutions at the sugar and/ or phosphate and/or base positions of a given nucleic acid sequence. See, e.g., U.S. Patent Application Serial No. 08/117,991, filed September 9 8, 1993, entitled High Affinity Nucleic Acid Ligands Containing Modified Nucleotides, now abandoned (see U.S. Patent No. 5,660,985), which is specifically incorporated herein by reference. Other modifications are known to one of ordinary skill in the art. Such modifications may be made post-SELEX (modification of previously identified modified or unmodified ligands) or by incorporation into the SELEX process.

At page 30, please replace the paragraph beginning at line 9 with the following:

Biotinylated TGF β 1 was prepared by reacting TGF β 1 at 3.6 μ M with an 11 fold molar excess of sulfo-NHS-biotin (Pierce, Rockford, IL, USA) in 50mM NaHCO₃ for 3 hr. in an ice bath. The reaction was acidified with 0.036 volumes of 10% acetic acid and applied to a 40 ~~mg~~ mg Vydac (The Separations Group, Hesperia, CA, USA) reverse phase column made in a siliconized pipet tip to separate unreacted biotin from biotinylated TGF β 1. The column was prewashed with 200 μ l ethanol followed by 200 μ l 1% acetic acid, the biotinylation reaction was applied, free biotin was washed through with 200 μ l of 50 mM sodium acetate pH 5.5, followed by 200 μ l of 20% acetonitrile and finally eluted with 200 μ l of 60% acetonitrile. The sample was lyophilized and resuspended in 50 mM sodium acetate pH 5.0 at 40 μ M and stored at 4° C. The TGF β 1 was spiked with 100,000 cpm iodinated TGF β 1 in order to follow recovery and to assess the success of the biotinylation reaction by measuring the fraction of the radioactivity that would bind to streptavidin coated agarose beads

(Pierce) before and after biotinylation. An aliquot of the TGFβ1 before and after biotinylation was subjected to analytical reverse phase chromatography. The biotinylated TGFβ1 substantially ran as a single peak which was retarded with respect to the unbiotinylated TGFβ. A small amount (5 %) of unreacted TGFβ1 could be detected. The efficiency of binding of the iodinated, biotinylated TGFβ1 to streptavidin (SA) agarose beads (30μl) was 30 % under the binding conditions used for SELEX partitioning.

At page 31, please replace the paragraph beginning at line 15 with the following:

SELEX ligands that bind to TGFβ1 were derived essentially as described in U.S. Patent No. 5,270,163 (see also, Tuerk and Gold (1990) Science 249:505-510). To generate the starting pool of PCR template, PCR product from twenty separate PCR reactions each containing 16.1 pmol of unpurified, single stranded DNA (at least a total of 2×10^{12} to 2×10^{13} different molecules) were pooled before the first transcription. PCR conditions were 50 mM KCl, 10 mM Tris-HCl ~~HCl~~ pH 9, 0.1% Triton-X100, 1.5 mM MgCl₂, 0.2 mM of each dATP, dCTP, dGTP, and dTTP, 2μM each primer and 0.075 units/μl of Taq DNA polymerase, 100 μl per reaction in a siliconized microfuge tube. All PCR cycles took advantage of hot start using Ampliwax (Perk and Elmer, Norwalk, CN, USA). Duration of the initial PCR was 10 cycles; a PCR cycle was 94° C-1', 52° C-1', 72° C-2'. An initial denaturation was 94°C for 4' and the final extension at 72°C for 5'. PCR reactions were combined, phenol/ chloroform extracted, and isopropanol precipitated (2.0 M ammonium acetate, 50% isopropanol) to remove primers.

At page 32, please replace the paragraph beginning at line 14 with the following:

TGFβ1-RNA complexes were separated from unbound RNA by washing the beads. Recovery of the selected 2'-NH₂ or F pyrimidine modified RNA from the agarose beads required guanidine thiocyanate extraction (5M GnSCN, 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.0, 0.1M beta mercaptoethanol) or from Seradyne SA coated beads by 2% SDS (0.1 M Tris-HCl pH 7.5, 50 mM NaCl, 1 mM Na₂EDTA, 2% SDS, 1.5mM DTT). Regular 2'-OH RNA was easily recovered under less harsh conditions with the same buffer used for the Seradyne beads containing only 0.2% SDS. After extraction and precipitation to purify and concentrate the RNA, the sample was reverse

transcribed with a cloned MMLV RT with the RNase H sequence deleted. The reaction contained less than or equal to 16 nM RNA, 10 μ M 3' primer, 50 mM Tris-~~HCL~~ HCl pH 8.3, 75 mM KCl, 5 mM MgCl₂, 10 mM DTT, 0.5 mM dNTP's. Prior to addition of buffer the RNA and the primer were boiled together. After addition of buffer and salts the reaction was annealed for 10 min at 28°C before addition of 600 units of Superscript reverse transcriptase (Bethesda Research Labs, Gaithersburg, MD, USA) and synthesis at 50°C for 1 hour.

At page 41, please replace the paragraph beginning at line 14 with the following:

Lib2-6-1 (SEQ ID NO:35) was shown to be fully effective only against TGF β 1 and not TGF β 2 and TGF β 3. Lib2-6-1 (SEQ ID NO:35) was biologically active in the presence of 10% horse serum in the cell culture medium in addition to the 0.1% BSA. Thus the ligand demonstrates specificity towards TGF β 1 which is not ~~interfered~~ interfered with by the presence of the horse serum in this assay. The biggest indication that the inhibition of TGF β 1 receptor binding is a specific phenomenon is the fact that not all TGF β 1 ligands block receptor binding, but the ones that do, do so reproducibly. There are no examples of ligands that do not bind to TGF β 1 blocking TGF β 1 receptor binding activity.

At page 44, please replace the paragraph beginning at line 11 with the following:

SELEX ligands that bind to TGF β 1 were derived essentially as described in U.S. Patent No. 5,270,163 (see also Tuerk and Gold (1990) Science 249:505-510) using the oligonucleotides illustrated in Table 5 (SEQ ID NOS:43-54). The DNA templates contained a 40-nucleotide (40N) variable sequence generated by mixed-nucleotide DNA synthesis, as well as 5'- and 3'-fixed sequences, necessary for PCR amplification of the template. The 5'-fixed sequence of oligonucleotides 40N7 (SEQ ID NO:43) and 40N8 (SEQ ID NO:49) also contained a T7 RNA polymerase promoter. RNA for the first round of RNA SELEX was transcribed from double-stranded DNA templates generated by primer extension on single-stranded DNA templates 40N7 and 40N8 with the Klenow fragment of DNA polymerase I. RNA SELEX consisted of up to 15 rounds of RNA synthesis, binding to target, partitioning of bound and unbound RNA by nitrocellulose filtration, cDNA synthesis, and PCR amplification to regenerate the double-stranded DNA template. Binding

to the target by the RNA pool was performed in binding buffer A (120 mM NaCl, 2.5 mM KCl, 0.12 mM ~~MgSO₄~~ MgSO₄, 40 mM HEPES, 20 mM ~~NaH₂PO₄/Na₂HPO₄~~ NaH₂PO₄/Na₂HPO₄ pH 7.4, 0.01% HSA) at 37°C degrees for at least 10 min prior to filtration. In contrast, the first round of single-stranded DNA SELEX was performed by using the synthetically synthesized oligonucleotides 40D7 and 40D8 directly. SELEX consisted of 25 rounds of binding to target, partitioning of bound and unbound single-stranded DNA by nitrocellulose filtration, PCR amplification to generate a double-stranded DNA population, and preparative polyacrylamide gel electrophoresis to purify single-stranded DNA for the next round of SELEX. Binding of the target to the single-stranded DNA pool was performed in binding buffer B (150 mM NaCl, 10 mM Tris-acetate pH 7.5, 0.001% BSA) at 37°C degrees for at least 15 min prior to filtration. Radiolabeling of RNA as well as DNA repertoires was performed by incubation of 5 picomoles nucleic acid, 2 units of T4 polynucleotide kinase, and 6 mL [γ ³²P] ATP (800 Ci/mmol) in a volume of 10 mL at 37°C degrees for 30 min. The concentration of nucleic acid at each round of the SELEX experiment varied between 1500 nM and 1 nM while the concentration of the target TGF- β 1 varied between 150 nM and 0.03 nM.

At page 46, please replace the paragraph beginning at line 9 with the following:

Binding analysis of the 40D7 DNA library for ~~TGF- β 1~~ TGF- β 1 is shown in Figure 1. Binding data obtained from round 19 (triangles) and round 0 (circles) are shown. The experiment was performed by incubating nucleic acid (less than 1 nM) and the indicated concentration of ~~TGF- β 1~~ TGF- β 1 in Binding Buffer (150 mM NaCl, 10 mM Tris-acetate pH 8.2, 0.001% BSA) for 15 minutes at 37°C degrees in a volume of 0.1 mL. Samples were filtered through nitrocellulose and were immediately followed by 3 mL of TE Buffer (10 mM Tris-acetate pH 8.0, 0.1 mM EDTA). The percentage of radiolabel bound was calculated from the amount of radiolabel retained on the nitrocellulose filter and the total radiolabel added to the binding reaction. The results show that the apparent *K_d* of the 40D7 library is 1 nM, whereas the starting pool has an apparent *K_d* of 30 nM. Thus, the 40D7 library shows an increase of about three fold in binding.

At page 46, please replace the paragraph beginning at line 21 with the following:

A PAI-luciferase assay to detect ~~TGF- β 1~~ TGF- β 1 activity in the presence of the nucleic acid

libraries generated in Example 5 was performed as described in Abe *et al.* (1994) Analytical Biochem. 216:276-284. Mink lung epithelial cells containing the PAI-luciferase reporter gene were incubated with ~~TGF- β 1~~-TGF- β 1 (10 pM) and oligonucleotides from the DNA libraries or anti-TGF-~~B~~-TGF- β antibody (60 μ g/mL). The mink lung epithelial cells were incubated for 18 hours and oligonucleotides were pre-incubated with ~~TGF- β 1~~-TGF- β 1 before the assay and read after 8 hours. Addition of oligonucleotides alone (100 nM) to the cell culture did not affect the assay (data not shown). The identity of the oligonucleotide libraries as well as their effect on luciferase activity is indicated in Figure 2. The ssDNA library 40N7 completely inhibited the activity of ~~TGF- β 1~~-TGF- β 1, while the control (an equal concentration of randomized nucleic acid) showed a small stimulation of ~~TGF- β 1~~-TGF- β 1 activity.

At page 63, please replace the paragraph beginning at line 10 with the following:

Recombinant human Keratinocyte Growth Factor (hKGF) and human Epidermal Growth Factor (hEGF) were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). haFGF, hbFGF, PDGF-AB, TGF β 1, and anti-KGF neutralizing monoclonal antibody were purchased from R&D Systems (Minneapolis, MN). Recombinant rat KGF was purchased from QED Advanced Research Technologies (San Diego, CA). Human thrombin was purchased from Enzyme Research Laboratories (South Bend, IN). T4 DNA ligase, *Hpa*II methylase, and restriction enzymes were purchased from New England Biolabs (Beverly, MA). pCR-Script Amp SK(+) cloning kit was purchased from Stratagene (La Jolla, CA). AMV reverse transcriptase was purchased from Life Sciences (St. Petersburg, FL). Taq DNA polymerase was purchased from Perkin Elmer (Foster City, CA). Ultrapure nucleotide triphosphates were purchased from Pharmacia (Piscataway, NJ). α -³²P-ATP, γ -³²P-ATP, and 5'-³²P-cytidine 3', 5'-bis (phosphate) (5'-³²P-pCp) were from DuPont NEN Research Products (Boston, MA). ¹²⁵I-labeled KGF was prepared as described before (Bottaro *et al.*, (1990) J. Biol. Chem. 265:12767-12770). PC-3 prostatic carcinoma cells were obtained from ATCC (catalog number CRL1435). Balb/MK cells and NIH3T3 transfected cells with the human KGF receptor (NIH3T3/KGFR) were a generous gift from S. Aaronson, Mt. Sinai Medical Center, NY, and have been described elsewhere (Miki *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89:246-250; Miki *et al.*, (1991) Science 251:72-75; Weissman *et al.*, (1983) Cell 32:599-606). T7 RNA

polymerase, 2'¹⁴NH₂- and 2'-F-modified CTP and UTP were from NeXstar Pharmaceuticals, Inc. (Boulder, CO). DNA oligonucleotides were obtained from Operon Technologies, Inc. (Alameda, CA). Nitrocellulose/cellulose acetate mixed matrix, 0.45 µm, HA filters were from Millipore (Bedford, MA). Calcium and magnesium containing Dulbecco's Phosphate Buffered Saline (DPBS) was purchased from Life Technologies (Gaithersburg, MD). Chemicals were at least reagent grade and purchased from commercial sources.

At page 64, please replace the paragraph beginning at line 8 with the following:

The SELEX procedure has been described in detail in US patent 5,270,163 (see also Tuerk and Gold (1990) Science 249:505-510). A single-stranded DNA (ssDNA) pool was used to generate the double-stranded (dsDNA) template for generating the initial random sequence RNA pool by transcription. The DNA template contained 40 random nucleotides, flanked by 5' and 3' constant regions for primer ~~annealing~~annealing sites for PCR and cDNA synthesis (Table 14; SEQ ID NOS:186-188). The 5' primer contains the T7 promotor sequence for *in vitro* transcriptions. The template was PCR amplified following an initial denaturation at 93°C for 3.5 minutes through 15 cycles of 30 second denaturation at 93°C, 1 minute annealing at 60°C, and 1 minute elongation at 72°C, in 50 mM KCl, 10mM Tris-HCl, pH9, 0.1% Triton X-100, 3 mM MgCl₂, 0.5 mM of each dATP, dCTP, dGTP, and dTTP, 0.1 units/µl Taq DNA polymerase, and 2.5 nM each of 3G7 and 5G7 primers (Table 14; SEQ ID NOS.187-188). SELEX experiments for hKGF were initiated with a random sequence pool of RNA in which all pyrimidines were 2'-NH₂-modified or 2'-F-modified. Transcription reactions were done with about 5µM DNA template, 5 units/µl T7 RNA polymerase, 40mM Tris-HCl (pH8), 12 mM MgCl₂, 5mM DTT, 1mM spermidine, 0.002% Triton X-100, 4% PEG 8000, 2-4 mM each 2'OH ATP, 2'OH GTP, 2'¹⁴NH₂-2'¹⁴NH₂ or 2'-F CTP, 2'¹⁴NH₂ or 2'-F UTP, and 0.25 µM α ³²P 2'OH ATP (800 Ci/mmmole). The full length transcripts were gel-purified prior to use. To prepare binding reactions, the RNA molecules were incubated with recombinant hKGF in Dulbecco's Phosphate-Buffered Saline (DPBS) with calcium and magnesium (Life Technologies, Gaithersburg, MD, Cat. No 21300-025) containing 0.01% human serum albumin. Following incubation at room temperature (ranging from 10 minutes to 10 hours) the protein-RNA complexes were partitioned from unbound RNA by filtering through nitrocellulose. Nitrocellulose filter bound

RNA was recovered by phenol/urea extraction. The partitioned RNA was reverse transcribed into cDNA by AMV reverse transcriptase at 48°C for 60 min in 50 mM Tris-HCl pH8.3, 60 mM NaCl, 6 mM Mg(OAc)₂, 10mM DTT, 50 pmol DNA 3' primer (Table 14), 0.4 mM each of dATP, dCTP, dGTP, and dTTP, and 1 unit/ μ l AMV RT. The cDNA was PCR amplified and used to initiate the next SELEX cycle.

Marked up version showing changes to claims under 37 C.F.R. § 1.121(c).

34. (amended) ~~The A method of claim 32 wherein said nucleic acids comprise modified nucleic acids~~ identifying modified single-stranded ribonucleic acid ligands to PDGF comprising:

a) contacting a candidate mixture of modified single-stranded ribonucleic acids with PDGF, wherein ribonucleic acids having an increased affinity to PDGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity ribonucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity ribonucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to PDGF, whereby modified ribonucleic acid ligands of PDGF may be identified.

56. (amended) ~~The A method of claim 55 wherein said nucleic acids comprise modified nucleic acids~~ identifying modified single-stranded ribonucleic acid ligands to hKGF comprising:

a) contacting a candidate mixture of modified single-stranded ribonucleic acids with hKGF, whereby ribonucleic acids having an increased affinity to hKGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity ribonucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity ribonucleic acids to yield a mixture of ribonucleic acids enriched for ribonucleic acid sequences with relatively higher affinity and specificity for binding to hKGF, whereby modified ribonucleic acid ligands of hKGF may be identified.